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Molecular analysis of variability among genotypes of *Abrus precatorius* L. with different seed coat colours using RAPD and ISSR markers

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Three genotypes of a medicinal, climbing herb *Abrus precatorius* L. (Family: Fabaceae), having different seed coat colour (red, black and white), were subjected to molecular analysis using PCR based RAPD and ISSR markers. Twenty RAPD (decamers) and fourteen ISSR primers were screened for their amplification potential. Of which 12 RAPD and 5 ISSR primers produced clear and reproducible amplified products. These primers yielded a total of 149 amplified fragments with an average of 8.76 bands per primer including 14 (9.39%) polymorphic fragments. In the study, ISSR fingerprinting (14.28%) detected more polymorphic loci as compare to RAPDs (7.89%). The data analyses based on Jaccard's similarity coefficient and UPGMA cluster analysis revealed that genotypes with black and white seed coats were more diverse as compared to genotype with red seed coat. Combined data of RAPD and ISSR further revealed that *Abrus* with white seed coat was more closely related to those having red seed coat.

Keywords: *Abrus precatorius*, genotypes, genetic variability, ISSR, molecular markers, RAPD

Abrus precatorius L. commonly known as 'Chirmi' or 'Rati' is a medicinal plant belonging to family Fabaceae. It is a climbing, perennial herb, which grows wild in the dry regions at low elevation. Plant parts (leaves, roots and seeds) are used in traditional medicines to treat wounds, scratches, sores, diabetes, chronic nephritis and arthritis[1]. Red seeded genotype is reputed for its anti-tumor[2] and anti-inflammatory properties[3], whereas white seeded genotype is used against leucoderma, leprosy, ulcer and tumour[4]. Medicinal properties of this plant are attributed to the glycoprotein known as lectins/agglutinins. In *A. precatorius*, three common genotypes based on seed coat colour (red, black and white) have been reported[5].

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Molecular markers (RAPD, ISSR, RFLP, AFLP and ISSR) have been demonstrated to be effective indicators for the assessment of genetic variation related to phenotypic traits. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) are widely employed techniques in the detection of genetic variations since they have several advantages, such as, technical simplicity, quick to perform, require small amount of DNA, the cost per assay is low, and no previous knowledge about the sequence is required. RAPD and ISSR techniques were successfully applied for assessment of genetic variability in a number of plant species[6,7]. The present investigation was undertaken to study the polymorphism among three genotypes of *A. precatorius* using RAPD and ISSR markers.

Three genotypes of *A. precatorius* L. having different seed coat colour (black, white and red) were selected for the present investigation (Fig. 1). Fresh and young leaf samples were collected from the field grown plants, maintained in the Field Gene Bank of the University College of Science, Mohanlal Sukhadia University, Udaipur, India. Total genomic DNA was

Fig. 1—*A. precatorius* L.: Field grown plant (A); Pods of *A. precatorius* (B); & Seeds of three genotypes—Red (C), Black (D) & White (E).

extracted from the leaves of the three genotypes of *A. precatorius* using cetyl trimethyl ammonium bromide (CTAB) method with minor modifications. The impurities of RNA were removed by the treatment of RNase A. The quality of genomic DNA was checked by agarose gel electrophoresis on 0.8% agarose gel (w/v), stained with 0.5 µg/mL ethidium bromide. DNA concentration was estimated spectrrophotometrically (UV-Vis Spectrophotometer, Pharmaspec UV-1700, Shimadzu, Japan) by measuring the absorbance at 260 nm. All the PCR reactions were carried out in 0.2 mL polypropylene PCR tubes (Bangalore Genei, India) using Thermal Cycler (Eppendorf). RAPD profiles were produced through PCR amplification using the protocol described by Williams *et al*[8] with minor modifications. Each 20 µL reaction mixture contained 1× Taq buffer (100 m*M* Tris-Cl *p*H 9.0, 500 m*M* KCl, 15 m*M* $MgCl_2$ and 0.1% gelatin), 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs (Bangalore Genei, India), 20 *p*mol oligonucleotide primers (Sigma Genosys, India), 1 U Taq DNA polymerase (Bangalore Genei, India) and 25 ng template DNA. All reactions were subjected to initial denaturation at 94°C for 4 min, followed by 30 amplification cycles, each consisting of 1 min at 94°C (denaturation step), 1 min at 37°C (annealing step) and 2 min at 72°C (extension step), with a final extension of 7 min at 72°C.

For ISSR analysis each 20 µL reaction mixture contained 1× Taq buffer (100 m*M* Tris-Cl *p*H 9.0, 500 m*M* KCl, 15 m*M* $MgCl_2$ and 0.1% gelatin), 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs (Bangalore Genei, India), 10 *p*mol oligonucleotide primers (Sigma Genosys, India), 1 U Taq DNA polymerase (Bangalore Genei, India) and 25 ng template DNA. Thermal cycler was programmed with an initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 sec denaturation at 94°C, 45 sec annealing at respective annealing temperature (45-60°C) and 90 sec extensions at 72°C. A final 10 min hold at 72°C ensured full extension of all amplifications products.

All the amplified products were separated on 1.5% agarose (w/v) gel, stained with 0.5 µg/mL ethidium bromide. 1 Kb DNA ladder (Bangalore Genei, India) and 100 bp DNA ladder (Bangalore Genei, India) were mixed and used as mol wt marker for comparison of amplified products. Gels were photographed under UV light using a Gel Documentation System (DP 001. FDC, Consort, Belgium). All reactions were repeated thrice to confirm the results.

For RAPD and ISSR analyses, 20 arbitrary decamer primers (RUF201-RUF220; Sigma Genosys, India) and 14 primers (IUF series; Sigma Genosys, India), respectively were used for screening, and only those primers were selected for the present study which provided satisfactory and reproducible amplification products under similar conditions (Table 1). Amplified fragments were scored as '1' or '0' for presence or absence of bands

Table 1—Details of RAPD and ISSR bands generated in *A. precatorius* genotypes

No.	Primer	Sequence (5'- -3')	Total no. of amplified fragment	No.of polymorphic bands	% polymorphism	Fragment size range (bp)
1	RUF 202	TTGGCGGCCT	9	1	11.11	450-1600
2	RUF 203	TTGGCGGCCT	15	1	6.66	250-1800
3	RUF 205	TGGGTCCCTC	10	0	0.00	350-2600
4	RUF 207	CAGGCCCTTC	10	0	0.00	300-1200
5	RUF 210	TGCCGAGCTG	12	3	25.00	600-6000
6	RUF 211	GGGTAACGCC	7	0	0.00	750-1600
7	RUF 215	GCTGCGTGAC	10	2	20.00	420-2000
8	RUF 216	CAGCGAACTA	8	1	12.50	480-2200
9	RUF 217	CGACTCACAG	9	0	0.00	400-1500
10	RUF 218	GGGCCTCTAT	11	0	0.00	490-1500
11	RUF 219	CTAGAGGTCC	9	1	11.11	660-1800
12	RUF 220	GGGTGAACCG	4	0	0.00	500-800
13	IUF016	(ACTG)4	9	2	22.2	420-1400
14	IUF017	(GACAC)4	3	0	0.00	900-1300
15	IUF019	$(AG)_{10}G$	6	1	16.66	600-2000
16	IUF021	$(AG)_{10}C$	10	0	0.00	590-19000
17	IUF022	$(AG)_{10}T$	7	2	28.57	500-1000
Total/Average			149	14	9.39	

on the gel. DNA polymorphism was calculated and given as percentage of the total number of bands produced in RAPD and ISSR profiles. RAPD and ISSR analyses were assembled and used in the statistical analysis. Jaccard's similarity coefficient was calculated to obtain genetic variability among three genotypes of *A. precatorius*. A dendrogram was constructed based on UPGMA cluster analysis.

In the present investigation, RAPD and ISSR profiles of three genotypes of *A. precatorius* were obtained. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. A total of 17 (12 RAPD and 5 ISSR) primers produced clear and reproducible amplified products. These primers yielded a total of 149 amplified fragments with an average of 8.76 bands per primer, including 14 polymorphic fragments. Among 17 primers, 9 (6 RAPD & 3 ISSR) gave polymorphic (9.39%) banding pattern (Fig. 2), while 8 (6 RAPD & 2 ISSR) produced monomorphic bands (Fig. 3). Most of the polymorphism was because of the absence of one or two bands. The size of the polymorphic bands ranged from 250 bp (RUF203) to 6000 bp (RUF210). In the present study, ISSR fingerprinting (14.28%) detected more polymorphic loci as compare to RAPD (7.89%).

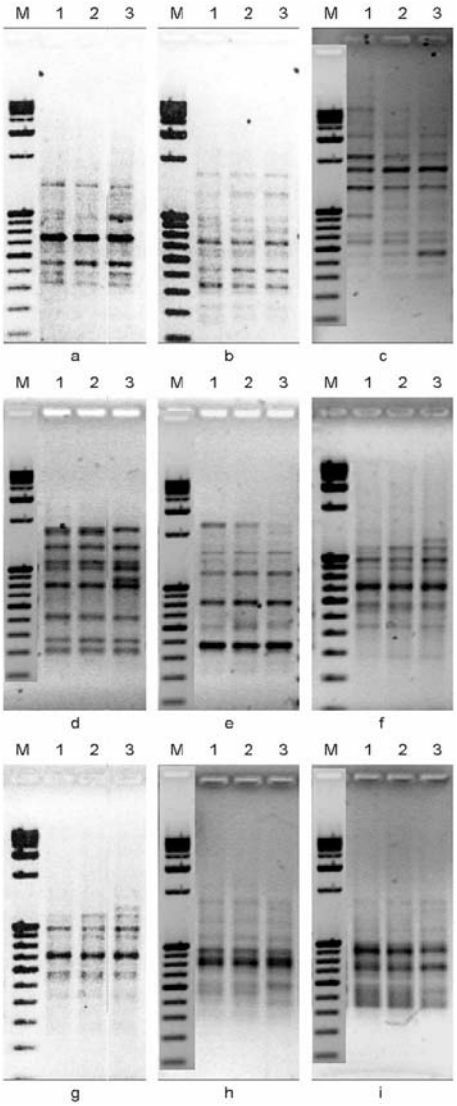

Fig. 2—RAPD and ISSR profiles of *A. precatorius* genotypes showing polymorphic banding patterns using selected primers (a. RUF202, b. RUF203, c. RUF210, d. RUF215, e. RUF216, f. IUF016, g. RUF219, h. IUF019, & i. IUF022). [M, Mol wt marker 1 Kb ladder; lane 1, White seeded; 2, Red seeded; & 3, Black seeded]

Jaccard's similarity coefficient values ranged from 0.9060 to 0.9860 (Table 2). The similarity matrix was subjected to UPGMA clustering to generate a dendrogram. Lowest Jaccard's similarity value represents maximum diversity. Genotypes with black and white seed coat were found to be more diverse as compared to genotype with red seed coat (Fig. 4). According to the combined results of RAPD and ISSR, white and red seed coat genotypes were the most closely related with the highest similarity index (0.98), whereas black genotype showed minimum similarity index with white genotype (0.90).

In conclusion, the results obtained during the present investigation provided evidence for the occurrence of genetic variability among three genotypes of *A. precatorius*. Both the marker systems

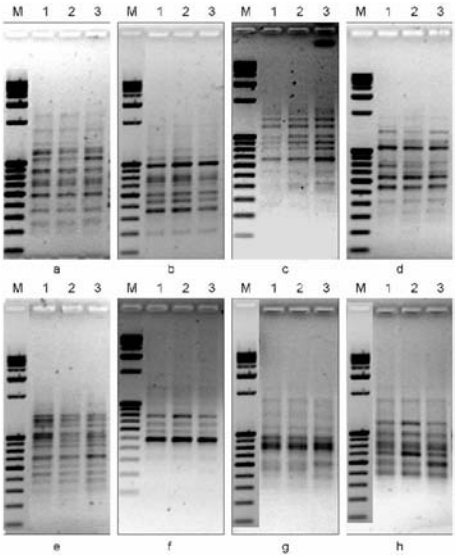

Fig. 3—RAPD and ISSR profiles of *A. precatorius* genotypes showing monomorphic banding patterns using selected primers (a. RUF205, b. RUF207, c. RUF211, d. RUF217, e. RUF218, f. RUF220, g. IUF017, & h. IUF021). [M, Mol wt marker 1 Kb ladder; lane 1, White seeded; 2, Red seeded; & 3, Black seeded).

Table 2—Jaccard's similarity coefficient values of *A. precatorius* genotypes

	White	Red	Black
White	1.0000		
Red	0.9860	1.0000	
Black	0.9060	0.9183	1.0000

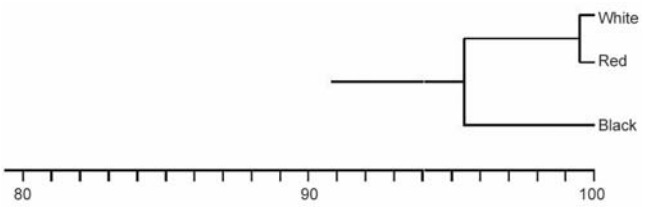

Fig. 4—Dendrogram showing relationship among *A. precatorius* genotypes (obtained from RAPD and ISSR analyses using UPGMA).

either individually or in combination have been successfully used to determine genetic variability in a number of plant species[9]. The level of polymorphism revealed by ISSR (14.28%) was higher compared to RAPD (7.89 %). Different markers might reveal different classes of variations[10], which might be correlated with the genome fractions surveyed by each kind of marker and its distribution throughout the genome[14]. In the present investigation, RAPD and ISSR profiles indicated very low degree of genetic variability which might occur due to several reasons. In case of *Maconopsis*[11], *Padicularis furbishae*[12] and *Oroxylum indicum*[13], low genetic variation has been reported to be due to their reproductive strategies, such as, selfing and vegetative propagation. This low level of genetic variability may be attributed to the restricted distribution in a particular area, less possibility of introgressions during evolution, non-effective gene flow, local selection pressure and inbreeding system[14]. The analysis of polymorphic bands could prove highly useful in identification of trait-linked genes in the genotypes determining seed coat colour.

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